The Association of Anti-Aminoacyl-Transfer Ribonucleic Acid Synthetase Antibodies in Patients With Rheumatoid Arthritis and Interstitial Lung Disease

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ABSTRACT

Objectives: This study aims to analyze the distribution and clinicopathological characteristics of anti-aminoacyl-transfer ribonucleic acid (tRNA) synthetase (ARS) antibodies in rheumatoid arthritis patients.

Patients and methods: We retrospectively studied the anti-ARS antibody levels in 228 RA patients' (44 males, 184 females; mean age 62.9±14.0 years; range 23 to 88 years) sera from their medical charts. We determined the association with anti-cyclic citrullinated peptide antibody levels, interstitial lung disease (ILD), rheumatoid factor, and methotrexate or biological disease modifying antirheumatic drug treatments.

Results: Anti-ARS antibodies were detected in 14 RA patients (6.1%). ILD complications were significantly higher among anti-ARS antibody-positive patients (57.1% vs 22.4%, p<0.05). Levels of anti-threonyl-tRNA-synthetase (anti-PL-7) and anti-alanyl-tRNA-synthetase (anti-PL-12), two anti-ARS antibodies, were higher in RA patients with concurrent ILD (both p<0.05). Myositis and ILD worsening were not observed in three anti-ARS antibody-positive patients despite biological disease modifying antirheumatic drug administration. There was no difference in anti-cyclic citrullinated peptide and rheumatoid factor specificities between patients with or without ARS antibodies.

Conclusion: Anti-ARS antibodies were detected in RA patients, with higher prevalence in patients with concurrent ILD. RA patients, specifically those with ILD complications, should be tested for anti-ARS antibodies.

Keywords: Aminoacyl-transfer ribonucleic acid synthetase; antirheumatic drugs; interstitial lung diseases; rheumatoid arthritis.
in this study, we aimed to analyze the distribution and clinicopathological characteristics of anti-ARS antibodies in rheumatoid arthritis (RA) patients.

**PATIENTS AND METHODS**

The study included a total of 228 RA patients (44 males, 184 females; mean age 62.9±14.0 years; range 23 to 88 years) who visited the Juntendo University Hospital between April 2010 and March 2014. Standards proposed by the 2010 American College of Rheumatology/European League Against Rheumatism were used for the diagnosis of RA.13 Autoimmune conditions were diagnosed based on the classification criteria for myositis.14 and ILD was confirmed by histopathology or by the detection of interstitial infiltrates after chest radiography (X-ray generator, 0.6/1.2 P324DK-125, Shimadzu Corporation, Kyoto, Japan, and X-ray photoreceiver, flat panel detector CALNEO-U, Fujifilm Medical, Tokyo, Japan) or high-resolution computed tomography (CT, Aquilion ONE TSX-301C/3A, Toshiba Medical Systems Corporation, Otawara City, Tochigi-ken Japan). The study protocol was approved by the Juntendo University Hospital Ethics Committee. A written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Anti-ARS antibodies were assessed using the line blot test kit (Myositis Profile Euroline Blot test kit, Euroimmun AG, Lübeck, Germany). The following antibodies were assayed: anti-OJ (anti-isoleucyl-tRNA synthetase; anti-EJ (anti-glycyl) antibody against glycyl-tRNA synthetase; anti-threonyl-tRNA synthetase (anti-PL7) antibody against threonyl-tRNA synthetase; anti-alanyl-tRNA synthetase (anti-PL-12) antibody against alanyl-tRNA synthetase; and anti-Jo-1 antibody against histidyl-tRNA synthetase. The manufacturer’s protocols were followed for each measurement. Sera from 14 patients who tested positive for anti-ARS antibodies were screened for anti-cytoplasmic antibody using indirect immunofluorescence test and commercially available human epithelial type 2 monolayer slides (Medical & Biological Laboratories, Co., Ltd., Nagoya, Japan). The sera were diluted 1:100 in phosphate-buffered saline (PBS) and incubated at room temperature for 45 minutes. After incubation, the slides were rinsed in PBS, followed by incubation with fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G (KPL, Gaithersburg, MD, USA) diluted 1:50 in PBS as a secondary detecting reagent, for 45 minutes at room temperature. After washing with PBS, the staining pattern was examined.

A commercial second-generation enzyme-linked immunosorbent assay kit (Immunoscan RA anti-cyclic citrullinated peptide [anti-CCP] test kit; Euro Diagnostica, Malmö, Sweden) was used to detect anti-CCP antibodies, following manufacturer’s instructions. Anti-CCP antibody was measured by the laboratory test company (SRL Inc., Hino-shi, Tokyo, Japan; test code 3301D). Briefly, serum samples were transferred to microtiter plates after a 1:50 dilution, and incubated at room temperature for 60 minutes. After washing, the plates were incubated with peroxidase-conjugated anti-human immunoglobulin G antibody at room temperature for 30 minutes. Samples were incubated with 3,3′, 5,5′-tetramethylbenzidine substrate solution at room temperature and the reaction was stopped with phosphoric acid after 30 minutes. Optical density measurements were conducted in a plate reader (Multiskan MS, Labsystems, Helsinki, Finland) at 450 nm. Each plate contained pre-diluted anti-CCP standards, and positive and negative controls. Anti-CCP antibody levels in serum samples were analyzed by a four-parameter curve fit. Samples with values exceeding 25 U/mL were considered positive for anti-CCP antibodies.

Rheumatoid factor (RF) was measured using a semi-quantitative latex agglutination test. A commercial kit (RapiTex RF; Behring, Marburg, Germany) was used following the manufacturer’s protocol. Serum titers greater than 1/4 (80 IU/mL) were considered RF-positive.

**Statistical analysis**

Fisher’s exact test was applied to analyze statistical significance between groups using SPSS version 10 software (SPSS Inc., Chicago, IL, USA). P values of <0.05 were considered statistically significant.
RESULTS

Table 1 shows the demographics of the 228 RA patients included in this study. Anti-ARS antibodies were detected in 14 patients (6.1%). Six of these patients had anti-PL-7 antibodies (2.6%); four had anti-EJ antibodies (1.8%); two had anti-PL-12 antibodies (0.9%); one had anti-OJ antibodies (0.4%); and one had anti-Jo-1 antibodies (0.4%). No patient tested positive for two or more anti-ARS antibodies. Anti-nuclear indirect fluorescence test revealed cytoplasmic staining with sera of patients positive for ARS (Figure 1), suggesting their anti-ARS specificity.

Data were stratified as anti-ARS antibody-positive and anti-ARS antibody-negative (Table 2). No statistically significant difference was noted between anti-ARS antibody-negative and anti-ARS antibody-positive patients in terms of age, sex, methotrexate (MTX) administration frequency, biological disease modifying antirheumatic drugs (bDMARDs) administration frequency, patients with concurrent interstitial lung disease (ILD) (Table 2). No statistically significant difference was noted between anti-ARS antibody-negative and anti-ARS antibody-positive patients in terms of age, sex, methotrexate (MTX) administration frequency, biological disease modifying antirheumatic drugs (bDMARDs) administration frequency, or the presence of ILD. Interstitial lung disease.

Table 1. Demographics of rheumatoid arthritis subjects (n=228)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
<td>62.9±14.0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>184</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTX administration</td>
<td>134</td>
<td>58.8</td>
<td></td>
</tr>
<tr>
<td>bDMARDs administration</td>
<td>96</td>
<td>42.1</td>
<td></td>
</tr>
<tr>
<td>Patients with concurrent ILD (%)</td>
<td>56</td>
<td>24.6</td>
<td></td>
</tr>
<tr>
<td>ILD, MTX-treated</td>
<td>20</td>
<td>35.7</td>
<td></td>
</tr>
<tr>
<td>ILD, bDMARDs-treated</td>
<td>17</td>
<td>30.4</td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard deviation; MTX: Methotrexate; bDMARDs: Biological disease modifying antirheumatic drugs; ILD: Interstitial lung disease.

Figure 1. Anti-nuclear antibody (indirect fluorescence) test. Test was performed using human epithelial type 2 to verify cytoplasmic reactivity of anti-aminoacyl-transfer ribonucleic acid synthetase antibodies. Patients 1-6 had anti-PL-7 antibodies (a-f); patients 7-10 had anti-EJ antibodies (g-j); patients 11 and 12 had anti-PL-12 antibodies (k and l), and patients 13 and 14 had anti-OJ (m) and anti-Jo-1 (n) antibodies, respectively.
(bDMARDs) administration frequency, RF values, and anti-CCP levels. However, ILD complications were significantly more frequent in the anti-ARS antibody-positive group than in the negative group (57.1% vs. 22.4%, p<0.05). To understand the effect of ILD on the prevalent antibody type, data were further stratified with respect to patient ILD status (Table 3). The incidence of anti-ARS antibody positivity was significantly higher in the ILD group (14.3% vs. 3.5%, p<0.05) and a significantly higher percentage of RA patients with ILD tested positive for anti-PL-7 antibody and anti-PL-12 antibody than non-ILD RA patients. No statistically significant differences were noted for other antibodies.

Table 4 contains detailed information (anti-ARS antibody status, age, sex, MTX and bDMARDs treatments, anti-CCP, and RF values) for patients

**Table 2.** Comparison of anti-aminoacyl-transfer ribonucleic acid synthetase antibody-positive and -negative patients

<table>
<thead>
<tr>
<th></th>
<th>Anti-ARS antibody-positive</th>
<th>Anti-ARS antibody-negative</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Female</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>Interstitial lung disease (+)</td>
<td>8</td>
<td>57.1</td>
</tr>
<tr>
<td>Methotrexate (+)</td>
<td>4</td>
<td>28.6</td>
</tr>
<tr>
<td>bDMARDs (+)</td>
<td>3</td>
<td>21.4</td>
</tr>
<tr>
<td>Rheumatoid factor (U/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid factor-positive rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-CCP (U/mL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ARS: Aminoacyl-transfer ribonucleic acid synthetase; SD: Standard deviation; NS: Not statistically significant; bDMARDs: Biological disease modifying antirheumatic drugs; Anti-CCP: Anti-cyclic citrullinated peptide.

**Table 3.** Anti-aminoacyl-transfer ribonucleic acid synthetase antibodies in rheumatoid arthritis patients according to interstitial lung disease status

|                                | Patients with concurrent ILD (%) (n=56) | Patients without concurrent ILD (%) (n=172) |
|                                | n  | %   |                  | n  | %   |                  | p   |
| Anti-ARS antibody (+)          | 8  | 14.3|                  | 6  | 3.5 |                  | <0.05|
| Anti-PL-7 antibody             | 4  | 7.1 |                 | 2  | 1.2 |                 | <0.05|
| Anti-EJ antibody               | 2  | 3.6 |                 | 2  | 1.2 |                 | NS  |
| Anti-PL-12 antibody            | 2  | 3.6 |                 | 0  | 0.0 |                 | <0.05|
| Anti-OJ antibody               | 2  | 3.6 |                 | 1  | 0.6 |                 | NS  |
| ILD: Interstitial lung disease; ARS: Aminoacyl-transfer ribonucleic acid synthetase; NS: Not statistically significant.

**Table 4.** Anti-aminoacyl-transfer ribonucleic acid synthetase antibody-positive rheumatoid arthritis patients with interstitial lung disease

<table>
<thead>
<tr>
<th>Patients</th>
<th>Anti-ARS</th>
<th>Age/Sex</th>
<th>MTX</th>
<th>bDMARDs</th>
<th>Anti-CCP (U/mL)</th>
<th>RF (U/mL)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>PL-7</td>
<td>63/M</td>
<td>-</td>
<td>-</td>
<td>364</td>
<td>1419</td>
</tr>
<tr>
<td>2</td>
<td>75/F</td>
<td>2 mg/W</td>
<td>-</td>
<td>ABA</td>
<td>198</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>54/F</td>
<td>-</td>
<td>-</td>
<td>0.6</td>
<td>228</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>PL-12</td>
<td>37/M</td>
<td>-</td>
<td>ABA</td>
<td>349</td>
<td>18.4</td>
</tr>
<tr>
<td>5</td>
<td>51/F</td>
<td>-</td>
<td>ETN</td>
<td>319</td>
<td>1110</td>
<td>127</td>
</tr>
<tr>
<td>6</td>
<td>75/F</td>
<td>-</td>
<td>-</td>
<td>5.6</td>
<td>727</td>
<td>135</td>
</tr>
</tbody>
</table>

ARS: Aminoacyl-transfer ribonucleic acid synthetase; MTX: Methotrexate; bDMARDs: Biological disease modifying antirheumatic drugs; Anti-CCP: Anti-cyclic citrullinated peptide; RF: Rheumatoid factor; EJ: Anti-glycyl; ABA: Abatacept; ETN: Etanercept.
who tested positive for anti-ARS antibody and had also ILD. MTX was administered to one patient while bDMARDs were administered to three patients. Abatacept and etanercept were administered to two and one patients, respectively. Except for one patient who tested positive for anti-PL-7 antibody, anti-CCP antibody and RF titers were high. Chest CT revealed that these patients did not have PM/DM during the clinical course of the disease (Figure 2). Chest CT scans revealed non-specific interstitial pneumonia lung complications in all patients. No characteristic changes were associated with the respective anti-ARS antibodies detected in patient sera. Moreover, patients with rapid progression of lung complications were absent.

**DISCUSSION**

Our study revealed the presence of anti-ARS antibodies in sera of 6.1% RA patients in our cohort; 57.1% of anti-ARS antibody-positive RA patients had concomitant ILD. This is a very significant result since, generally, anti-ARS antibodies are detected in PM/DM patients but not in RA patients. Notably, RA patients testing positive for anti-ARS antibodies did not differ from RA patients testing negative for these antibodies in terms of age, sex, RF positivity, and anti-CCP antibody positivity. However, the incidence of ILD complications was significantly higher in RA patients who were positive for anti-ARS antibodies in comparison with patients who tested negative, and CT scan findings from all ILD patients were suggestive of non-specific interstitial pneumonia.

Anti-ARS antibodies are known as myositis-specific autoantibodies and have been extensively detected in idiopathic inflammatory myopathies and in patients with inflammatory myopathies overlapping with RA. Bernstein et al. have reported anti-Jo-1 antibodies in the sera of 25% of PM/DM patients, 7.5% of myositis patients without the complication of ILD, 68% of patients with ILD complication, and 3% of patients with ILD in the absence of myositis. In another study, anti-Jo-1 antibodies have also been reported in 15-30% of PM patients and 60-70% of ILD patients. Other anti-ARS antibodies are less predominant in inflammatory myopathies. In our study, however, anti-Jo-1 antibodies was detected in only one RA patient, whereas anti-PL-7 antibody was detected in six patients (42.9% of all anti-ARS antibody-positive patients). Early diagnosis of these antibodies has been associated with a better overall survival.

In RA patients with concomitant ILD, PL-7, PL-12, and EJ antibodies were detected, with PL-7 antibodies having the highest incidence. Targoff et al. reported a correlation between anti-EJ antibody
incidence and ILD in PM patients, and established that these antibodies reacted with glycyl-tRNA synthetase. However, anti-PL-7, anti-PL-12, anti-OJ, anti-Zo (anti-phenylalanyl), and anti-KS (anti-asparaginyl) antibodies were reported to have a stronger correlation with ILD than that between anti-Jo-1 antibody and DM/PM patients.\(^{22-27}\) In our population of RA patients, anti-PL-7 antibodies were most frequently detected in patients who had ILD complications (four); PL-12 and EJ antibodies were each detected in two patients. Notably, however, the anti-PL-12 antibody was detected only among ILD patients. Kalluri et al.\(^ {28}\) reported that 90% of patients who tested positive for anti-PL-12 antibodies also had ILD. They have concluded that the presence of anti-PL-12 antibodies is more strongly associated with ILD than with myositis and arthritis.\(^ {25}\)

Biological disease modifying antirheumatic drugs, such as tumor necrosis factor, interleukin-6, or cytotoxic T-lymphocyte associated protein 4, are recommended for the treatment of RA and they play an important role in improving the patient’s activities of daily living and quality of life.\(^ {28}\) Recent reports suggest that anti-tumor necrosis factor-alpha treatment of patients with elevated anti-ARS antibody levels may trigger myositis.\(^ {29}\) However, our results show that even in RA patients testing positive for anti-ARS antibodies and who were administered bDMARDs, connective tissue disorder complications such as PM/DM or transition/progression were absent. Although ILD complication was observed in 57% of RA patients who had autoantibodies against ARS, treatment with MTX and bDMARDs could be safely and effectively administered. Moreover, rapid progression of ILD was not seen in myositis patients testing positive for anti-ARS antibodies.\(^ {30}\)

Our study has certain limitations. First, being a retrospective study, the comprehensiveness of data collection could not be ascertained; second, this is a single-center study, which might be subject to regional bias and limit our sample size. Additional multicenter large-scale prospective studies are required to establish the anti-ARS antibody profile of RA patients. Furthermore, as we were unable to analyze the levels of anti-ARS antibodies in healthy subjects, a more controlled study is desired. We have, however, found that patients with other diseases such as Sjögren’s syndrome and microscopic polyangiitis are negative for anti-ARS antibodies. In spite of these limitations, the results of the study are important because, for the first time, they provide strong evidence of anti-ARS antibodies in RA patients, with a different distribution between ILD-positive and -negative patients.

In conclusion, line blot analysis of anti-ARS antibody levels in RA patients revealed that these autoantibodies were present in 6% of RA patients. Autoantibodies, particularly anti-PL-7 and anti PL-12 antibodies, were detected in a significantly higher percentage in patients with the complication of ILD. No significant effects of bDMARDs on the incidence of connective tissue disorder complications/transition/progression were observed in anti-ARS antibody-positive RA patients. RA patients, specifically those with ILD complications, should be tested for Anti-ARS antibodies. Ultimately, we hope that the results of this study will have therapeutic and diagnostic implications and will stimulate further research in this important area.

**Declaration of conflicting interests**

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**REFERENCES**


